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HUBR-1186 (10102735)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	Dony, et. al.
Serial No.	:	09/806,635
Filed	:	June 4, 2001
For	:	USE OF A MELANOMA INHIBITING ACTIVITY FACTOR (MIA) FOR CARTILAGE AND BONE REPAIR
Art Unit	:	1646
Examiner	:	J. L. Andres

August 1, 2003

This is to certify that this correspondence is being sent by facsimile transmission addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below

Eileen Sheffield

Commissioner for Patents
P. O. Box 1450
Alexandria, VA. 22313-1450

Sir:

RULE 132 DECLARATION OF INVENTOR

1. I am a co-inventor of the above referenced patent application. I am fully familiar with the patent application and the office actions which have issued in the course of its prosecution, including the office Action dated March 25, 2003.

2. I submit this declaration with the accompanying data and references in view of the examiner's analysis of the application, particularly the Examiner's 35 U.S.C. § 112, first paragraph rejection of claims 18, 23, 24 and 33.

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3. Attached are experimental data for Example 5 of the instant specification which was conducted under my direction. The data show that after 14 days implants were isolated and underwent histological analysis. Implants with increasing amounts of MIA protein were larger in overall size and histological analysis revealed highly stimulated cartilage formation in these specimens. The extent of cartilage formation was directly linked to the concentration of MIA applied to the implants. This can be seen in Figs. 1 and 2 of the data.

4. Given the directives of Example 5 and the data attached hereto, it is my opinion that the rejected claims are enabled.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

By _____
Dr. Carola Dony

Attachment

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Example 5

Mouse bioassay for cartilage, bone, tendon and ligament induction

Similar to the Sampath and Reddi rat ectopic implant assay, a mouse ectopic implant assay, using outbred NMRI mice, 4 months old was performed (Sampath and Reddi, Proc. Natl. Acad. Sci. USA 80 (1983) 6591-695; WO 95/16035). (a) MIA alone, (b) BMP-2 alone and (c) combinations of MIA and BMP-2 were applied in the appropriate buffer, 0.1% trifluoroacetic acid for BMP-2 and 100 mM potassium-phosphate, 150 mM NaCl, pH 6.0 for MIA. As carrier were used collagen type I matrix and hyaluronic acid. Any suitable carrier maybe used, e.g. collagen type I matrix, collagen-heparin mixture, gelatine capsules, hyaluronic acid, alginate or other functionally equivalent device, based on biocompatibility, biodegradability, stability and mechanical properties.

The implants were placed intramuscular into the gluteus muscle of the mouse and left for 14 days. After 14 days the mice were sacrificed by cervical dislocation. The implants were isolated and processed using standard histological techniques (see Theory and Practice of Histological Techniques, ed. Bancroft and Stevens, Churchill Livingstone, 1996). Paraffin sections (4-7 µm) were cut and stained with von Kossa/Toluidine blue to visualize and quantitate the amount of cartilage and bone tissue induced in each implant. Positive (e.g. BMP-2) and negative (e.g. mock device) implant control groups were compared to experimental implants.

To assess the quality of cartilage and/or bone induced, gene expression can be studied by RNA in situ hybridization for cartilage and bone markers as described above, using cartilage markers (e.g. collagen II, collagen X) and bone markers (e.g. collagen I, osteocalcin).

After 14 days implants were isolated and underwent histological analysis. Implants containing osteoinductive factor alone were smaller in size and only bone with bone marrow and fibrous tissue could be detected in the histological sections. No cartilage tissue could be detected (fig. 1B). In contrast, implants with increasing amounts of Mia protein were bigger in overall size and histological analysis revealed highly stimulated cartilage formation in these specimens (fig. 1D). The extent of cartilage formation was directly linked to the concentration of Mia applied to the implants (fig. 2). In implants with more than 10 µg Mia (combined to 1 µg of the osteoinductive protein BMP2) very high extent of cartilage tissue was detected, resulting from prolonged cartilage formation rate and inhibition of bone formation. No bone formation was detectable in these implants.

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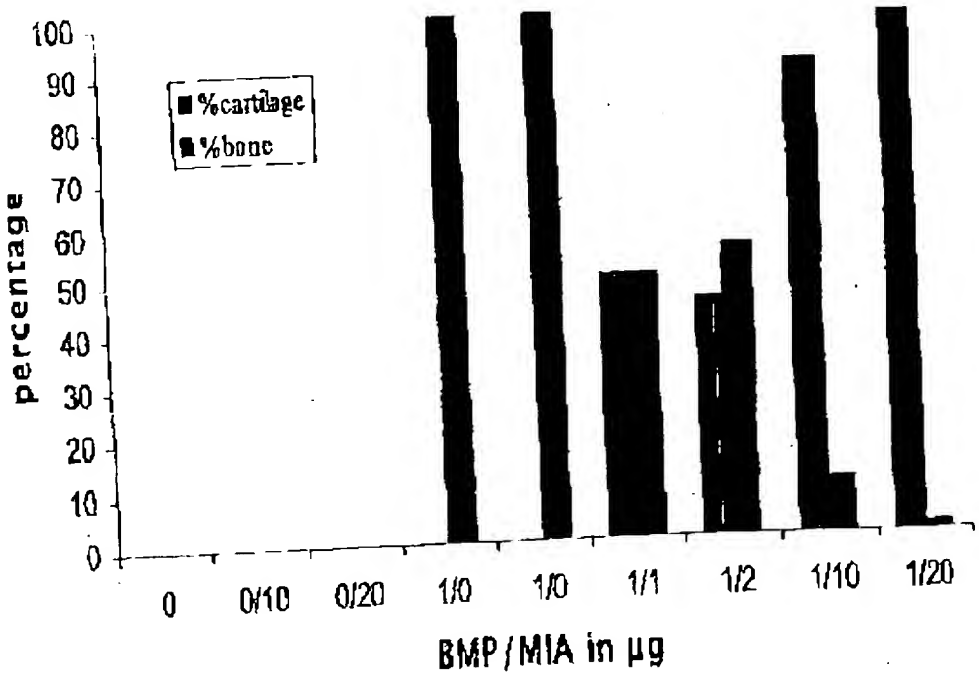
Figure 1

fig. 2

BMP2 & MIA Ectopic Bone Assay in Mouse



matrix: hyaluronic acid; time: 14 days

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Experiments were carried out under the supervision of:

Dr. Gabriele Prötzel

Dr. Carola Dony